Supplemental Material: The role of the extracellular matrix protein mindin in airway response to environmental airways injury

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Running Title: Mindin regulates environmental airways disease

Keywords: ozone, lipopolysaccharide, LPS, endotoxin, TLR4, toll-like receptor, innate immunity, lung

Acknowledgements: This work was supported by the NIH (ES016126 to JWH and HL081825 to JPE).

SUPPLEMENT METHODS

RT-PCR

Total RNA was extracted from whole lung homogenates using Qiagen RNeasy Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's protocol. RNA samples were then treated with RNase-free DNase I (Ambion, Austin, TX) to clear DNA contamination. cDNA was synthesized using BIO-RAD iScript reverse transcriptase (IBio-Rad Laboratories, Hercules, CA) following the manufacturer's instruction. All real-time quantitative polymerase chain reactions (qPCRs) were performed using ABI SDS 7300 and SYBR advantage qPCR premix (Clontech, Mountain View, CA). Primers were produced by IDT, Inc. (Coralville, IA) and sequences are as follows:

TLR4 Forward, 5'-AGAAATTCCTGCAGTGGGTCA-3';

TLR4 Reverse, 5'-TCTCTACAGGTGTTGCACATGTCA-3';

Mindin Forward, 5'-CAGCCCTGACTGGTTTGTGGGC-3';

Mindin Reverse, 5'-CCCTGGGACTCTGCTGTAGCCGCACG-3';

18S Forward, 5'-GTAACCCGTTGAACCCCATT-3';

18S Reverse, 5'-CCATCCAATCGGTAGTAGCG-3'.

HA Challenge

As previously reported (Garantziotis, Li et al. 2009; Garantziotis, Li et al. 2010), sterile, endotoxin-free (0.00008 endotoxin units/mL) high molecular weight hyaluronan (HMW-HA) (Healon, AMO, Santa Ana, CA) was reconstituted at 0.5 mg/mL in 0.02 M acetate, 0.15 M sodium chloride, pH 6.0. For the production of low molecular weight hyaluronan

(sHA), hyaluronan was sonicated on ice. In some experiments, 50 μL of either sHA or vehicle were instilled oropharyngeally into anesthetized mice and AHR was measured invasively 2-4 h later.

Statistics

Data are expressed as mean ± S.E. Significant differences between groups were identified by Student's *t* test. A two-tailed *p* value of < 0.05 was considered statistically significant.

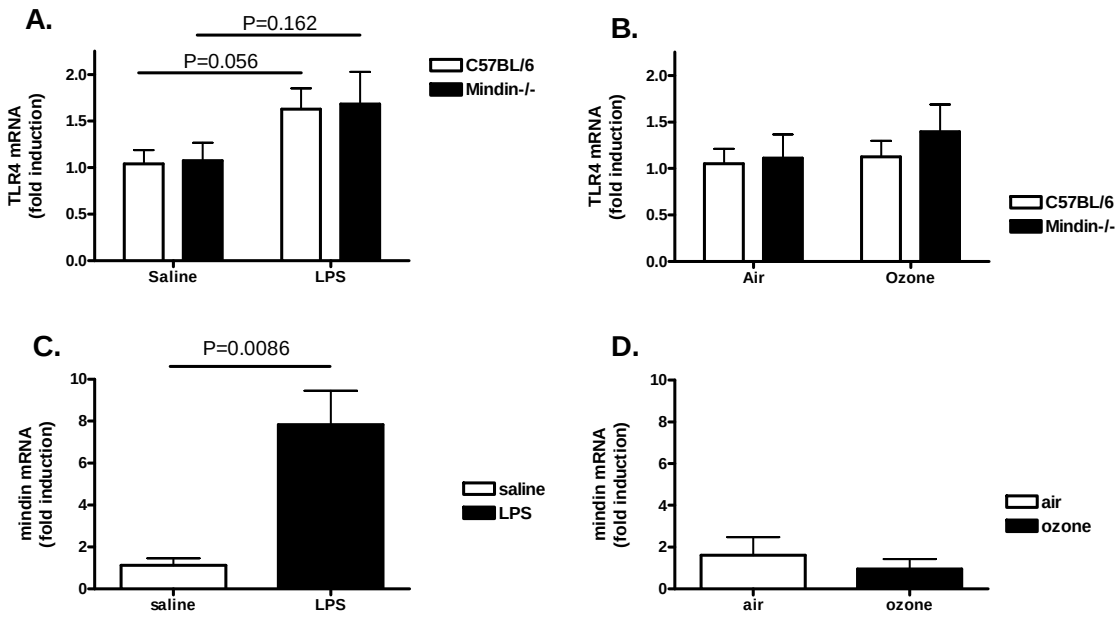
REFERENCES

Garantziotis, S., Z. Li, et al. (2009). "Hyaluronan mediates ozone-induced airway hyperresponsiveness in mice." J Biol Chem 284(17): 11309-11317.

Garantziotis, S., Z. Li, et al. (2010). "TLR4 is Necessary for Hyaluronan-mediated Airway Hyperresponsiveness After Ozone Inhalation." Am J Respir Crit Care Med 181(7): 666-675.

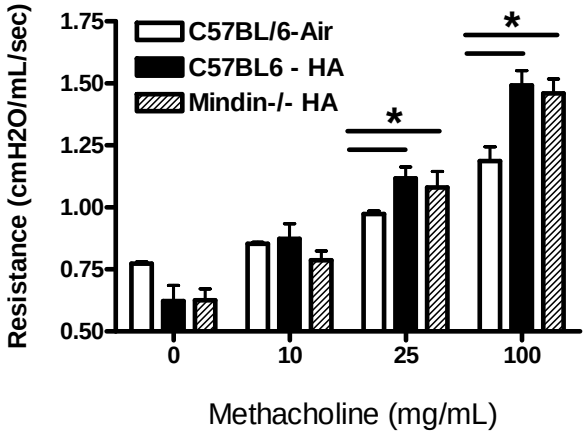
FIGURES

Supplement Figure 1.



Supplement Figure 1. *Whole lung TLR4 expression is independent of mindin and level of mindin expression after environmental challenge. (A)* TLR4 expression after exposure to saline and LPS. No significant differences in fold induction were observed, although there was a trend toward increased fold induction after LPS exposure in both WT (p = 0.056) and KO (p = 0.162) mice as compared to their saline-exposed counterparts. *(B)* TLR4 expression after exposure to filtered air and ozone. No significant differences in fold induction were observed. *(C)* Mindin expression was significantly increased after exposure to inhaled LPS when compared to saline (p = 0.0086). *(D)* Mindin expression was not affected by inhalation of ozone.

Supplement Figure 2.



Supplement Figure 2. *Enhanced methacholine sensitivity after challenge to hyaluronan is independent of mindin.* At low levels of methacholine (0 and 10 mg/mL, respectively), no significant differences in AHR were detected. At 25 mg/mL, there was a significant increase in AHR (*$p < 0.05$) between HA-exposed KO mice and air-exposed WT mice. There was also a significant increase (*$p < 0.05$) between HA-exposed WT mice and their air-exposed counterparts. These same statistically significant increases were also true at a methacholine dose of 100 mg/mL.